APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1995, p. 1653–1654 Vol. 61, No. 4
0099-2240/95/$04.00+0

A Simple Method for Selective Isolation of *Stenotrophomonas maltophilia* from Environmental Samples

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Received 12 September 1994/Accepted 27 January 1995

***Stenotrophomonas maltophilia* is a commonly found environmental bacterium that is associated with the plant rhizosphere. It shows increasing prevalence in immunocompromised patients. We report a simple method for selective isolation of *S. maltophilia* from soils which makes use of both its resistance to imipenem and its requirement for methionine.**

Stenotrophomonas maltophilia (formerly *Xanthomonas maltophilia*) (7) is a ubiquitous free-living bacterium. It can be isolated from water but is found more often in soils and especially in the plant rhizosphere (4). Debette and Blondeau have shown that this association is promoted by the high content of sulfurated amino acids in root exudates, which are growth factors for *S. maltophilia* (1).

In humans, *S. maltophilia* is an opportunistic pathogen (5). It is not a part of the normal flora of healthy humans, but it is frequently encountered as a commensal in the transient flora in hospitalized patients. *S. maltophilia* can cause severe disease in immunocompromised patients (6). In France, *S. maltophilia* has been isolated from clinical samples with increasing frequency since 1987, when imipenem, the first carboxypenem antibiotic, appeared. For years, this antibiotic remained active against *Pseudomonas aeruginosa* and other gram-negative bacilli, leading to wide use of imipenem in hospitals. However, *S. maltophilia* is naturally resistant to imipenem because it produces imipenemase (2). *S. maltophilia* is also resistant to most β-lactam antibiotics because it overproduces broad-spectrum β-lactamase. The increasing use of these antibiotics has led to the emergence of this bacterium in hospitalized patients. Some nosocomial transmissions have been described, but the origins of the strains remain unknown, in part because of the lack of a selective medium. However, in 1989 Juhnke and Des Jardin described a selective medium with a high recovery rate but two major disadvantages: low specificity (more than a third of the isolated strains did not belong to the species *S. maltophilia*) and tedious preparation (four basic components and eight antibiotics) (3). Here we report a simple method for selective isolation of *S. maltophilia* from soils which makes use of both its resistance to imipenem and its requirement for methionine.

Of the 34 soil samples studied, 8 were obtained in France from the rhizospheres of cruciferous plants (*Brassica sativa*, *Iberis sempervirens*, and *Diplotaxis erucoides*). The others, all from plant rhizospheres, were from different parts of the world (Vietnam, the People's Republic of China, Hong Kong, Morocco, and Ivory Coast). In all cases, 1 g of soil was placed in a tube containing 10 ml of nutrient broth (bioMérieux, Marcy-l'Etoile, France) with 0.5 mg of DL-methionine (Sigma Chemical Co., St. Louis, Mo.) per ml. After 24 h of incubation at 30°C, 0.1 ml of the broth was inoculated on a 90-mm-diameter Mueller-Hinton agar plate with a spreader (a bent Pasteur pipette). Within 15 min after inoculation, four disks impregnated with 10 μg of imipenem (bioMérieux) each were applied to the surface of the inoculated plates. After 18 h of incubation at 30°C, in order to obtain tiny colonies, colonies that grew in the inhibition areas of the disks were reisolated and identified by using the API 20E identification system (bioMérieux) according to the manufacturer's instructions.

By using this technique, we were able to isolate 21 imipenem-resistant strains. All were *S. maltophilia* strains. The strains were compared with clinical isolates by using the Biotype 100 auxanogram system (API bioMérieux, La Balme-les-Grottes, France). There was no difference in vigor or pattern (8) between the strains isolated from hospitalized patients and the strains isolated from soils.

To establish the sensitivity of this method, we inoculated tubes containing 1 g of sterile soil with 10-fold successive di-

TABLE 1. Sensitivity of the isolation method

CFU/g of soil	CFU/g on inhibition area for strain[a]:		
	A	B	C
0 (blank)	NC	NC	NC
10	80	10	NC
100	1,000	200	10
1,000	>1,000	1,000	80
10,000	>1,000	>1,000	1,000

[a] Data are means from two replicate experiments. NC, no colonies.

TABLE 2. Recovery of *S. maltophilia* from 10 different samples of soils from the Salvator Hospital gardens

Sample	CFU/g of soil[a]	
	Control	With inoculum of 100 CFU/g of soil
1	NC	>1,000
2	NC	100
3	125	>1,000
4	NC	50
5	NC	100
6	NC	125
7	NC	>1,000
8	NC	100
9	NC	>1,000
10	NC	>1,000

[a] Data are means from two replicate experiments. NC, no colonies.

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lutions of a suspension containing 4,000 CFU of *S. maltophilia*. To pinpoint interstrain variations, three strains isolated from different soils (strain A from France, strain B from Vietnam, and strain C from Morocco) were studied. Colonies that grew in the inhibition area were counted (Table 1). The sensitivity ranged from 10 to 100 CFU/g of soil, depending on the strain.

In order to evaluate the recovery rate of this method, 10 soil samples, obtained in Salvator Hospital's gardens far from any plant, were inoculated with 100 CFU of *S. maltophilia*. A control experiment without inoculation was performed to determine the possible presence of *S. maltophilia* in the sample. The results are presented in Table 2. It seems possible that sample 3 was contaminated with approximately 100 CFU of *S. maltophilia.*

The method described in this article is simple, specific, sensitive, and inexpensive. By using successive dilutions of the soil samples, one can obtain semiquantitative results. Conservation of the broth containing DL-methionine at 4°C is not needed, making it possible to perform experiments in the field.

We thank Jacqueline Debette, Roland Blondeau, and Juliette Charrel for helpful discussions.

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